ATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

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PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

Date of mailing *(day/month/year)*	24.06.2004
Applicant's or agent's file reference: 2948-177.PCT	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/US 03/21061	07.07.2003	08.07.2002

Applicant
EXPONENTIAL BIOTHERAPIES, INC. et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

The applicant's attention is drawn to Article 33(5), which provides that the criteria of novelty, inventive step and industrial applicability described in Article 33(2) to (4) merely serve the purposes of international preliminary examination and that "any Contracting State may apply additional or different criteria for the purposes of deciding whether, in that State, the claimed inventions is patentable or not" (see also Article 27(5)). Such additional criteria may relate, for example, to exemptions from patentability, requirements for enabling disclosure, clarity and support for the claims.

Name and mailing address of the international preliminary examining authority:	Authorized Officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Hutterer, G Tel. +49 89 2399-8066

ATENT COOPERATION TRL .TY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference 2948-177.PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US 03/21061	International filing date *(day/month/year)* 07.07.2003	Priority date *(day/month/year)* 08.07.2002
International Patent Classification (IPC) or both national classification and IPC A23L3/3571		
Applicant EXPONENTIAL BIOTHERAPIES, INC. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 6 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
10.01.2004	24.06.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Smeets, D Telephone No. +49 89 2399-7492

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US 03/21061

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1, 3-18	as originally filed
2, 2a	filed with telefax on 07.06.2004

Claims, Numbers

1-47	filed with telefax on 07.06.2004

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☐ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

   ☐ the entire international application,

   ☒ claims Nos. 19,20

   because:

   ☒ the said international application, or the said claims Nos. 19,20 relate to the following subject matter which does not require an international preliminary examination (specify):

   see separate sheet

   ☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

   ☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

   ☐ no international search report has been established for the said claims Nos.

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

   ☐ the written form has not been furnished or does not comply with the Standard.

   ☐ the computer readable form has not been furnished or does not comply with the Standard.

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-47
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-47
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-18, 21-47
	No:	Claims	

2. Citations and explanations

   see separate sheet

Re Item III

Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

For the assessment of the present claims 19 and 20 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following documents:

D1: Applied and Environmental Microbiology (1993), 59(9), 2914-2917
D2: Applied and Environmental Microbiology (07-2000), 66(7), 2951-2958
D3: DE-C-4326617
D4: Applied and Environmental Microbiology, Washington,DC, US (01-04-1996), 62(4), 1133-1140
D5: GB-A-2253859

Observations:

Claims 19 and 20 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

1. Novelty - Article 33(1) and (2) PCT; Inventive Step - Article 33(1) and (3) PCT

Independent claims 1, 15, 19, 21, 22, 34, 35 and 42 are novel as phage P100 and the endolysin protein derived from this phage are not derivable from any of the available prior art documents. Said phage is virulent against *Listeria monocytogenes* and can be used in bacterial control and detection methods.

Claims 2-14, 16-18, 20, 23-33, 36-41, 43-47 are dependent claims and as such also meet the requirements of the PCT with respect to novelty and inventive step.

The present invention concerns the use of a recently discovered Listeria phage with specific, essential and relevant properties, which makes it particularly suitable for identifying and controlling Listeria contamination of dairy products, facilities and equipment.

In addition to the general scientific literature on the subject, there is also patent literature that teaches the utility of phages in general to control bacterial contaminations in food processing plants and in foodstuffs. See for example U.S. Patent No. 5,006,347 issued on April 9, 1991, U.S. Patent No. 4,851,240 issued on July 25, 1989, GB 2 253 859 A published on September 23, 1992 and EP 0414304A2 published on February 27, 1991. However, none of the above discussed patents disclose a Listeria phage which was actually tested and shown to successfully control bacterial contamination in food processing plants and in food products. The reason for this is that all of the Listeria phages known in the art at the time of the disclosure in the previous patents were temperate phages, and were therefore not efficient at nor suitable for industrial bacterial eradication purposes. The term "temperate" refers to the fact when a strain of phage injects its DNA into a bacterial target, the phage DNA integrates into the DNA of the host cell, as a "prophage", and can remain integrated therein for considerable periods of time. Since the prophage excises (and initiates replication and lysis) only when the host cell becomes stressed, the ensuing bacterial lysis is unpredictable and not easily controlled, which is why temperate phages do not lend themselves well to industrial applications. Temperate phages are unsuitable for industrial decontamination purposes for other reasons as well, including the fact that they can deliver unwanted and dangerous genes to the bacteria target into which their DNA integrates. In contrast, there is a class of phages that lyse bacterial targets directly, given that they do not have the molecular machinery required to integrate into the bacterial targets. Such phages are referred to as being "virulent" or "lytic" for the bacterial targets. Virulent phages against Listeria monocytogenes were discovered recently, by one of the present inventors.

The first of these virulent Listeria phages, designated A511, was described in the literature in 1990 (see Loessner et. al., Applied and Environmental Microbiology, June 1990, p.1912-1918, 1990). See also DE 43 26617 C; Loessner et. al., Applied and Environmental Microbiology, April 1996, vol. 62, No. 4, p.1133-1140; and Gaeng et al., Applied and Environmental Microbiology, July 2000, vol. 66. No. 7,

p.2951-2958. The virulent phage according to the present invention belong to the Myoviridae family and have tails which contract towards the virus head. One particularly preferred phage is designated P100 and was deposited at the

We Claim:

1. A method for controlling Listeria contamination in a food product, on food processing equipment, or on food storage containers, comprising applying lytic phage P100, ATCC patent deposit designation no. PTA-4383, to a food product or food processing equipment in an amount sufficient to reduce the amount of Listeria.
2. The method according to claim 2, wherein said P100 is applied in combination with phage A511, ATCC patent deposit designation no. PTA-4608.
3. The method according to claim 1, wherein said lytic phage is applied in combination with at least one agent selected from the group consisting of listeriolysin, a surface disinfectant, an antibiotic, a surfactant, an enzyme, and a phage specific for bacterial contaminants other than Listeria monocytogenes.
4. The method according to claim 1, wherein said food product is a dairy product.
5. The method according to claim 1, wherein said food product is an unpasteurized food product.
6. The method according to claim 1, wherein said food product is a meat product.
7. The method according to claim 6, wherein said meat product is a ready to eat meat product.
8. The method according to claim 1, wherein said food product is a fish product.
9. The method according to claim 1, wherein said food storage container is a salad bar and said food product is salad.
10. The method according to claim 1, wherein said food processing equipment is selected from the group consisting of a tube through which milk is being pumped, a high-salt content tank for processing cheese, a container from which cultures are applied to a surface of a cheese, a set of shelves on which a product is dried and cured, and a floor drain.
11. The method according to claim 1, wherein said lytic phage are applied by mixing with a liquid or semi-solid food product.
12. The method according to claim 1, wherein said lytic phage are mixed with a liquid and sprayed onto a surface selected from the group consisting of food products, food processing equipment and food storage containers.

13. The method according to claim 12 wherein said lytic phage are applied to said food processing equipment in combination with an agent selected from the group consisting of listeriolysin, a surface disinfectant, an antibiotic, a surfactant, an enzyme, and a phage specific for bacterial contaminants other than Listeria monocytogenes.

14. The method according to claim 1, wherein said lytic phage are lyophilized or cryopreserved by vitrification and applied in a dry form to said food product, food processing equipment and food containers.

15. A composition comprising phage P100, ATCC patent deposit designation number PTA-4383 and a carrier.

16. The composition according to claim 15, further comprising phage A511, ATCC patent deposit designation number PTA-4608.

17. The composition according to claim 15, further comprising an agent selected from the group consisting of listeriolysin, a surface disinfectant, an antibiotic, a surfactant, an enzyme, and a phage specific for bacterial contaminants other than Listeria monocytogenes.

18. The composition according to claim 15, wherein said carrier is a pharmaceutically acceptable carrier.

19. A method for treating an animal infected with Listeria monocytogenes comprising administering an amount of P100 suitable to reduce or eliminate said Listeria monocytogenes.

20. The method according to claim 19, further comprising administering phage A511.

21. Phage P100 deposited at the American Type Culture Collection, ATCC patent deposit designation number PTA-4383.

22. A method for detecting the presence of Listeria monocytogenes, comprising
obtaining a sample suspected to contain Listeria monocytogenes,
incubating said sample with P100 according to claim 21, and
detecting any change in said sample caused by P100, as an indication of the presence of Listeria monocytogenes.

23. The method according to claim 22, wherein said change in said sample is due to lysis by P100 or a detectable label or signal.

24. The method according to claim 22, further comprising recombinantly inserting a gene construct into the genome of P100 before incubation with said sample, wherein expression of said gene construct results in a detectable signal in the presence of Listeria monocytogenes.

25. The method according to claim 24, wherein said gene construct encodes a bio-luminescent protein.

26. The method according to claim 25 wherein said bioluminescent protein is selected from the group consisting of luciferase and a fluorescent protein.

27. The method according to claim 26, wherein said luciferase is from bacteria or insects.

28. The method according to claim 26, wherein said fluorescent protein is green fluorescent protein or a variant thereof.

29. The method according to claim 22, further comprising immobilizing said Listeria monocytogenes on a solid support and detecting any change on said solid support.

30. The method according to claim 29, wherein said Listeria monocytogenes are immobilized using anti-Listeria antibodies.

31. The method according to claim 30, wherein said solid support is a test strip.

32. The method according to claim 22, wherein said sample is obtained from a patient suspected of being infected with Listeria monocytogenes.

33. The method according to claim 22, wherein said sample is obtained from a food product, food processing equipment or food storage containers.

34. A purified endolysin protein derived from phage P100.

35. A method for controlling Listeria contamination in a food product, on food processing equipment or on food storage containers, comprising applying the endolysin protein according to claim 34, to a food product, food processing equipment or food storage container in an amount sufficient to reduce the amount of Listeria.

36. The method according to claim 35, further comprising applying at least one variety of lytic phage from the Myoviridae family to said food product, food processing equipment or food storage container.

37. The method according to claim 35, wherein said lytic phage is selected from the group consisting of P100 and A511.

38. The method according to claim 35, wherein said endolysin is recombinantly produced.

39. The method according to claim 35, further comprising applying endolysin from at least one other phage which infects Listeria or another bacterial genera.

40. The method according to claim 39, wherein said other phage is A511.

41. The protein according to claim 34, wherein said endolysin protein is recombinantly produced.

42. A composition for controlling Listeria contamination in a food product, on food processing equipment or on food storage containers comprising endolysin protein derived from phage P100 according to claim 34, and a suitable carrier.

43. The composition according to claim 42, further comprising at least one variety of lytic phage from the Myoviridae family.

44. The composition according to claim 43, wherein said lytic phage are selected from the group consisting of P100 and A511.

45. The composition according to claim 42, wherein said endolysin is recombinantly produced in a host bacteria.

46. The method according to claim 22, wherein a gene construct has been recombinantly inserted into P100 in order to provide or emit a signal confirming the detection of Listeria monocytogenes.

47. The method according to claim 46, wherein said gene construct is selected from the group consisting of genes encoding luciferase and green fluorescent protein.